CLAIMS

What we claimed is:

1. A recombinant herpes simplex virus, characterized in that a DNA sequence is inserted in the genome, wherein said DNA sequence comprising a nucleotide sequence selected from the group consisting of the sequences represented by SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4 and SEQ ID NO: 5, or a homologous sequence thereof.

2. The recombinant herpes simplex virus according to claim 1, characterized in that the DNA sequence is inserted at XbaI site of the UL2 gene or UL44 gene of HSV genome.

3. The recombinant herpes simplex virus according to claim 1, characterized in that the DNA sequence represented by SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4 or SEQ ID NO: 5 is inserted in other nonessential gene regions of HSV genome.

4. A method for the production of a recombinant herpes simplex virus, comprising constructing DNA segments represented by SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4 or SEQ ID NO: 5 respectively, and inserting the five DNA segments into HSV genome using gene engineering method(s), respectively, thereby obtain the recombinant herpes simplex virus.

5. A recombinant herpes simplex virus according to claim 1, characterized in that the recombinant herpes simplex virus is inserted by other DNA sequences which is homologous to the DNA segment represented by SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4 or SEQ ID NO: 5.

6. A method for large-scale production and preparation of recombinant adeno-associated virus serotype 1, 3, 4, 5 or 6, characterized in that the method comprises the steps of:

(1) preparing and producing the recombinant herpes simplex viruses according to claim 1;

(2) establishing "a vector cell", namely, recombinant AAV vector cell strain;

(3) infecting corresponding vector cell strains respectively with the five recombinant herpes simplex viruses of (1); and

(4) producing a lot of recombinant adeno-associated viruses by vector cell strains infected respectively with the five recombinant herpes simplex viruses.

7. A method for isolation and purification of recombinant adeno-associated virus serotype 1, 3, 4, 5 or 6, characterized in that a crude lysis solution comprising recombinant adeno-associated virus-containing cells and the culture medium thereof is isolated and purified via the following steps:

1) adding chloroform to the crude lysis solution to deactivate HSV helper viruses, lyse cells, and denature and precipitate a great many cell proteins to obtain cell lysis solution;

2) adding solid sodium chloride to the cell lysis solution until the final concentration is 1.0 to 1.2 mol/L with stirring for dissolution, then centrifugating the mixture and leaving the supernatant;

3) precipitating rAAV with PEG/NaCl, adding solid polyethylene glycol to the sodium chloride-containing supernatant of step 2) with stirring for dissolution, letting the mixture sit, then centrifugating the mixture and discarding the supernatant but leaving the precipitate;

4) treating the cell lysis solution with DNaseI and RNase to degrade the nucleic acid, dissolving the precipitate of step 3), and adding DNaseI and RNase to dissolve the residual nucleic acid apart from the rAAV viral particles;

5) using chloroform to extract and remove other proteins and the residual PEG, adding chloroform to extract, and then, centrifugating the mixture and removing the upper water phase;

6) removing salts via dialysis; and

7) further purifying rAAV via density gradient centrifugation or affinity chromatography.

8. A recombinant vector plasmid pSNAV-NX, characterized in that the recombinant plasmid comprises ITRs at the two ends of AAV-1, AAV-3, AAV-4, AAV-5 or AAV-6 genome, with an immediate early enhancer and a promoter of cytomegalovirus, a multiple cloning site and a polyA signal successively intervening between the two ITRs, and a neomycin resistant gene expression cassette flanking the outside of the ITR.

9. The method for isolation and purification of recombinant adeno-associated virus serotype 1, 3, 4, 5 or 6 according to claim 7, characterized in that the method is useful for large-scale isolation and purification of the so-called "AVV empty capsid", i.e. AAV serotype 1, 3, 4, 5 or 6 without gene therein.

10. A method for purification of recombinant adeno-associated virus serotype 1, 3, 4, 5 or 6, characterized in that the method comprises: adjusting the conductance value of obtained rAAV solution before passing through an ion exchange column which has been balanced by a buffer, balancing the ion exchange column using a buffer again, then eluting the ion exchange column with a salt-containing buffer and collecting the elution peaks; passing the collected elution peaks through a molecular sieve column which has been balanced by a buffer, followed by washing the column with a buffer again.